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IMMUNITY AND ADAPTATION.[1]

LEO LOEB.

Various writers, in discussing the phenomena of adaptation, included among them the fact that in many cases organisms become immune against certain injurious influences, as for instance the action of toxic substances, provided that these influences were active during a sufficiently long period; the immunity against disease-producing bacteria was especially regarded as a phenomenon of adaptation. These writers, therefore, explained immunity by including it among the larger class of adaptive phenomena. Although this attitude appears from a certain point of view as the most rational one, it may nevertheless in other respects be more fruitful to adopt the opposite attitude, which consists in trying to gain an insight into the mechanism of certain adaptive phenomena, by making use of the discoveries made in the course of investigations in immunity. This attitude seems also justified by the fact that a large number of instances of acquired immunity cannot be directly explained as adaptive phenomena. In these respects, therefore, the conception of immunity is wider than that of adaptation.[2] The main reason for taking the second attitude, however, lies in the fact that the phenomena of immunity have been partially at least accessible to an experimental analysis, much more so than other phenomena of adaptation.

The latter may be conveniently classified as internal and ex-

[1] From the Pathological Laboratory of the University of Pennsylvania.

[2] T. H. Morgan in his book on "Evolution and Adaptation" includes the phenomena of immunity among the adaptive processes. He expresses, however, the view that certain of these phenomena could not be explained as due to any selective processes. The following remarks, the partly hypothetical character of which is quite apparent, were written with the aim of connecting a number of somewhat isolated facts, and of suggesting the possibility of an extension of such considerations to different fields of investigation.

ternal, as adaptations of one part of the body to another part of the same organism and adaptations of an organism or of one of its constituent parts to the outer world, although these two kinds of adaptations must not necessarily be regarded as sharply distinct, inasmuch as it can be assumed that external conditions cause a primary internal reaction, which gives rise to a secondary internal change, the latter possessing the character of an adaptation and therefore representing directly an internal, indirectly however an external adaptation.

It appears possible to connect certain characters, whose usefulness for the organism possessing them, seems to justify their classification among the adaptive phenomena with other facts which have been found by the experimental study of immunity. A few adaptations of this kind may be selected to demonstrate this connection. In the tissues of vertebrates and invertebrates, substances are present which have a strongly accelerating effect upon the coagulation of the blood. If a wound is made and the blood escapes, the contact with these tissues has a tendency to stop the bleeding and prevent the animal from bleeding to death. The tissues of each class of animals are especially adapted to the fibrinogen of their own blood. The blood of a bird coagulates more quickly under the influence of the tissue of a bird than of a dog or of a frog. The blood of a turtle coagulates more quickly under the influence of the tissues of a turtle than of the tissues of a mammal or of a bird. The same holds good of frogs' blood. Invertebrate blood, like that of a lobster, is not at all influenced by the tissues of vertebrates, but very powerfully so by the tissues of the lobster and to a less degree by the tissues of some other invertebrates. Here we have apparently to deal with a specific adaptation. A certain relationship exists between blood and tissues of one class of animals; a specific relationship, which proves very beneficial to the animal. A somewhat analogous fact is mentioned by Duclaux. He states that it is well known to manufacturers of dairy products that the milk of a certain species of animals is more rapidly coagulated by the rennin of the same than of another species.[1]

[1] Although the analogy between these two facts is clear, the usefulness apparent in the case of the tissue coagulins, seems to be absent in the case of the specificity of rennin. The latter fact can therefore not be included among the phenomena of adaptation.

Certain parasitic animals, as the leech and anchylostoma, live entirely or partially by taking into their digestive system the blood of the host. In regard to anchylostoma it is not quite certain whether it sucks the blood of the host directly or whether it obtains the blood together with parts of the mucous membrane of the internal canal which it inhabits. Blood of the host certainly forms an important part of the food of the parasite. Both of these worms contain in the anterior part of their body, substances which strongly inhibit the coagulation of the blood. Thus the blood is kept in a liquid state and the sucking of the blood and probably also its digestion and resorption are rendered much easier. This was demonstrated in the case of the leech many years ago by Haycraft and recently also in the case of anchylostoma. Here again we have to deal with a process which to some degree is specific, inasmuch as the substance which has such a powerful action in inhibiting the coagulation of the blood of the host is absolutely without influence on the coagulation of invertebrate blood. And there are even certain indications tending to show that this substance does not act equally strongly on all vertebrate blood, inasmuch as Sabbatani found that a similar substance in another blood-sucking animal, ixodes ricinus, acts much more strongly on dog blood, the blood which is sucked by this tick, than on rabbits' blood. I myself found in one experiment made that the substance which is present in anchylostoma, inhibiting the coagulation of the blood of the dog, was powerless towards the blood of guinea pigs.[1]

The fact that leech extract is without effect on the blood of the lobster makes it furthermore more probable that it is also without power upon the blood of the leech itself.

Snake venom, a most powerful poison for many vertebrates, is almost harmless for snakes, themselves. This is as Phisalix found at least partially due to the presence of an antitoxin in the blood of the snakes. The blood of these animals may therefore contain toxin and antitoxin side by side. This immunity of snakes against their own poison is of great significance, otherwise an injury of their tongue or any other part of their body by the teeth of the animal would be fatal.

A toxin similar to snake venom is contained in the abdominal

[1] This experiment needs to be repeated.

segments of scorpions. The scorpions possess, as Metschnikoff describes, in their blood an antitoxin against scorpion venom, which is very poisonous for other insects and to vertebrates. Certain desert animals are frequently exposed to the bite of scorpions, and in accordance with this fact, it has been recently found that such animals are not susceptible to this poison, although other animals nearly related to the desert animals, but not living in localities where they are exposed to bites of scorpions, are easily poisoned. Whether the blood of these animals has any antitoxic action, does not yet seem to have been determined.

For a long time an explanation had been sought for the fact that the mucous membrane is not digested by the pepsin and hydrochloric acid, which are secreted by the cells of the mucous membrane. Recently Weinland has shown that the cells of the mucous membrane of the stomach contain a substance able to neutralize the action of the digestive ferment, an antipepsin. Although it is not certain or perhaps even unlikely that the presence of this substance is sufficient to account in itself for the power of resistance shown by the cells of the mucous membrane, yet it represents in all probability at least one important contributing factor and is therefore a cellular adaptation, without which life would be impossible. [1]

In the course of the study of the action of bacteria and their toxins on the animal organism, it has been found that injection of bacteria into the body of an animal may cause in the serum of the injected animal the appearance of substances, which produce an agglutination or even a solution of the bacteria (agglutinins, bacteriolysins). Injections of bacterial toxins may produce the appearance of antitoxin in the serum. Later it was found that this response of the animal organism was not limited to bacteria and their products but was also present in the case of injections of animal and plant cells in general and even of ferments and albuminous substances. The substances which after a certain period are to be found in the blood serum have all this in common that in some way they antagonize the organisms or substances, which had been injected and which had caused the appearance of the reactive substances. The way in which this antagonizing effect does take place is different in different cases.

[1] Metschnikoff, "L'immunité," Paris, 1901. Chapter XI.

Some facts are known in regard to the place of origin of such "antibodies" and in regard to the conditions which favor or inhibit their appearance. From a certain point of view we may at present perhaps distinguish three types of reactions.

1. If at certain intervals a solution of abrin is dropped into the conjunctival sac of an animal, substances are formed in the conjunctiva to which the abrin was applied, which are able to neutralize the injurious effect of abrin. In this case a local response of the cells on which the substance acted, has taken place.

2. If we inject substances, as cultures of the cholera vibrio subcutaneously, intraperitoneally or intravenously, antibodies are produced in organs far removed from the place of injection, as for instance in the spleen and in the bone-marrow. The mere injection of these substances is sufficient to produce this result.

3. There exists a third type of reaction, which seems to be of a more complicated character than the forementioned ones, and which hitherto has been regarded as a totally different process, but which seems to be essentially of a similar character. The normal pancreas, which is the source of several hydrolytic ferments usually does not produce lactase, a ferment capable of splitting the disaccharid lactose. If, however, an animal receives with its food a certain quantity of lactose, for instance in the form of milk, lactase is secreted by the pancreas. The introduction of a carbohydrate into the animal body causes, therefore, in this case the appearance of a specific substance able to destroy the carbohydrate. The lactase may be called an antibody. If we inject, however, lactose directly into the blood, lactase is not produced. The mechanism of the production of this substance seems to have been in the main cleared up by Bayliss and Starling and Bainbridge. It appears that through the introduction of lactose into the alimentary canal a substance is formed in the mucous membrane of the small intestine, which gradually passes into the circulation and causes the pancreas to secrete the lactase. It is possible to extract this substance from the intestines in vitro and to cause a secretion of lactase by injecting the extract into a vein. In this case it is not the original substance itself which by passing into the circulation causes directly the appearance of an antibody, but it is a second substance formed under

the influence of the lactose in the cells, with which the lactose or component parts of the lactose comes into contact.[1]

The difference between the second and third mode of action is not of such a character as to render it impossible to regard the latter as related to the former, especially as we do not know whether intermediary processes do not also take place even in those reactions, which are included under the second type. It is not impossible that a similar complicated character will be shown to exist in some reactions in the second class, which have been regarded as direct ones.

The presence of antibodies can, however, not always be demonstrated in cases of artificially produced immunity. It has for instance been impossible to obtain antibodies after injection of certain chemical substances, which are of a less complicated character than the albuminous substances mentioned above, *e. g.* certain alkaloids.

If we now turn again to the adaptations, which were mentioned above as occurring naturally, it is not difficult to see that a great similarity exists between the natural and the experimental reactions. The existence of an antipepsin in the mucous membrane of the stomach seems analogous to the production of antiabrin in the conjunctiva. In both cases we have to deal with an apparently local production of an antibody. If we find in scorpions and in snakes an antibody against their own venom circulating in their blood, it is not unlikely that we have to deal with a reaction of the second class. The fact of the leech and ankylostoma producing substances inhibiting the coagulation of the kind of blood they swallow may with some probability be classed among the reactions of the third type. The same probably holds good for the tissue coagulins. Whether in the case of the desert animals an antibody is present does not seem to have been determined; it is, therefore, possible that we might have to deal with an acquired immunity without the presence of an antibody which can be demonstrated. On the other hand it is not unlikely that further investigation will show the existence of an antitoxin.

Plausible as the explanation given of some of these adaptations, as experiments of nature in immunity may appear, there seems

[1] The deductions of Starling and Bainbridge have recently been controverted by Bierry.

to be some objection to applying it to cases in which the substance causing the immunization or adaptation is derived from the same animal in which the antibody is produced. Antibodies have been experimentally produced only in such cases in which certain substances derived from one animal were injected into another animal and usually those belonging to a different species. The experimental proof has, however, been given that in certain cases iso-antibodies can be produced, by the injection of animal cells or animal products into other animals of the same species. Furthermore it could be shown that such antibodies may act not only on the cells of another animal of the same species but even on the analogous cells of its own body. This for instance was found in the case of the spermotoxins. An antibody against its own spermatozoa can be produced in the same animal which has been injected with spermatozoa of another animal of the same species. Whether the spermatozoa are able to produce antibodies if they are injected into the same animal from which they are derived, has apparently not yet been investigated; but it appears not unlikely that it might succeed. Such an experiment gave negative results in the case of the blood cells which are however normally circulating in the blood. Certain other facts point to the conclusion that such autoantibodies may be formed without experimental interference under natural pathological and even normal conditions. If one kidney becomes chronically diseased by experimental interference so that tissue of the kidney is being resorbed, the serum seems to assume properties, injurious to kidney tissue of the same animal. If we inject such serum into the circulation of another animal of the same species, albuminuria appears as a sign that the kidneys have been injured. Even if this observation should be open to a different interpretation, there are other facts which suggest a similar conclusion. It is certain that such digestive ferments as trypsin pass normally into the circulation and may be excreted by the kidney. As might be expected from what has been said before, an antitrypsin exists in the serum, or the serum has antitryptic properties, just as the snake blood contains antitoxin against snake venon.

Glaessner found even that the tryptic power of the serum varies

at different periods of the day and that these variations indicate a certain relationship to the time at which the food is taken in and at which trypsin is secreted. This is very suggestive of the rapid formation of an antibody in response to the normal increase of a certain ferment in the circulation. In a similar way the blood serum of a horse and a pig was found by Briot, Roden and Korschun to contain normally an antibody against rennin. This natural antibody acts in the same way as the one experimentally produced by injecting rennin into other animals, probably combining with the rennin. A further observation strengthening the evidence that autoimmunizatory processes may take place is that during the puerperium an increase of isoagglutinins takes place in the human body. This is probably the result of the resorption of substances from the uterus and other organs. The resorption of these substances seems to modify the action of the blood serum on the blood cells of the same species of animals.

The specific character of the experimentally produced antibodies is quite marked; these antibodies possess a certain relationship to that substance whose introduction into the animal organism caused the antibody to appear. This specificity is of a twofold character. In the first place the antibody reacts only against a chemical substance similar in composition to the one which caused it to originate, secondly there exists a species specificity. Substances apparently equally constituted, but derived from different species, behave differently towards the antibody, those substances showing the strongest reaction which are derived from the same species, or from a species similar to the one from which the substance was obtained whose introduction into an animal organism caused the formation of the antibody. The latter (and also the former) kind of specificity is not absolute, substances derived from nearly related species showing a similar although usually a weaker reaction.

Such a specificity may also be noticed in the adaptations described above. The snake and scorpion antitoxin are specific for snake and scorpion toxin respectively. The leech extract is without effect on the plasma of invertebrates, but acts only on the plasma of vertebrates; in the case of ixodes and anchylostoma, the specificity may perhaps even go farther, so that the

antiferment acts mainly on the blood of that species which is sucked by the parasite. In regard to the tissue coagulins, their specificity has already been mentioned above. We might be able to distinguish by the aid of this specificity for instance the muscle of a lobster from that of a blue crab. In the whole, however, we have to deal here rather with a class specificity than with a species specificity. The specificity is not so strongly pronounced in the case of the naturally produced antibodies as in those cases in which antibodies are produced artificially. Glaessner believes that a specificity of the antitrypsin of the blood exists even among different mammalians. The number of his experiments seems, however, to be very limited, so that at present this specificity cannot yet be accepted as proven. It is possible that also in this case a class specificity does exist rather than a species specificity. This may also apply to enterokinase, which activates trypsinogen. A species specificity does not seem to exist in this case, but a class specificity may nevertheless be present.

Both classes of substances, the experimentally produced, as well as the naturally occurring substances, are specific, not only in the sense that they are chemically different from substances found elsewhere but that they have a specific relationship to the substance which caused their appearance and that they indicate the species or class origin of this latter. Their action is, therefore, a selective one and they are not only specific in the sense of being chemically different from other substances but they are specifically adapted to a certain action on a very limited number of substances. We may, therefore, call these substances "specifically adapted" substances.

We suggested that the tissue coagulins owe their origin to a process of autoimmunization, the character of a certain fibrinogen determining the character of the coagulins of the same species or class. Tissue coagulins resemble very closely enzymes. The secretion of lactase, a typical ferment, is, as we saw, due to the introduction of lactose into the organism. This suggests that other ferments have a similar origin; they may be regarded as antibodies produced by a complex process of autoimmunization. The existence and interaction of ferments in the animal organism is perhaps the most perfect instance of internal adaptation.

So far we had only to deal with chemical not with structural adaptations. It seems, however, possible to extend the preceding considerations to structural adaptations. Ferments produce primarily chemical changes. But we know of chemical ferment actions which bring about structural changes in the medium in which they act. Thrombin in transforming fibrinogen into fibrin changes a colloidal fluid into a gelatinous more or less solid mass, which under the influence of pressure and traction may show a fibrillar structure not unlikely connective tissue. From a certain point of view, the fibrin ferment may, therefore, be regarded as a form producing ferment.[1] We might call it a morphogenic ferment. We have reason to assume that there exist other morphogenic ferments. So far mainly the splitting activity of ferments has been studied. It is not unlikely that the action of many enzymes may be reversible. There would then exist a large number of enzymatic actions, leading to the building up of complicated chemical compounds from relatively simple substances. Such an action would probably in part again be connected with the creation of definite structures, which would be different under the influence of different enzymes. These enzymes would have a specific species, and also a specific individual character and the analogous products created by such ferments would be different in different species and in different individuals.

Such an interaction of substances with the resulting formation of antibodies could especially be conceived of as taking place during embryonic development; it would tend to produce a correlation between different chemical and structural mechanisms which might in part at least account for the harmony which exists in the function and structure of different parts of an organism.

It has not yet been investigated as far as I am aware, whether some of the adaptations mentioned above have been acquired in each individual or whether they are hereditary. That might be doubtful, especially for instance in the case of the immunity of scorpions and snakes against their own venom. The latter alternative is, however, very likely in the case of the anticoagulative substance of the leech and of anchylostoma and in the case

[1] The fact that the fibrin ferment produces organized substance has already been noted by Gautier.

of the tissue coagulins. It might be of interest to examine the existence of such a substance in leeches which had never had an opportunity of sucking blood containing an active thrombin, *e. g.*, of leeches which had been nourished on blood which had been previously heated to 56°, and to investigate whether the desert animals raised at places where they are exempt from scorpion bites, would nevertheless develop immunity against scorpion bites.

If a hereditary fixation of the production of an antibody should take place, then such a substance could be formed independently of the presence of the substance which originally caused its production and the origin of such an adaptation would, if regarded as an isolated fact, not be apparent.

In connecting these different facts it was intended to show the analogy existing between several adaptations which are probably fixed by heredity and certain reactions of the animal organism which can be produced experimentally. If we shall be able to clear up more and more the phenomena of experimental immunization we may hope to explain by the same studies, one factor which seems to form an essential part of many adaptations.

Most of the reactions considered so far are useful for the organism in which the reaction is taking place. It is, however, possible to produce experimentally reactions of a similar character, in which no useful result can be recognized. It is furthermore not unlikely that the same principle (the formation of antibodies) may under certain naturally occurring circumstances lead to conditions which are injurious to the animal organism, as in the case of destructive processes taking place in one kidney. Uremia is explained by Ascoli through the formation of nephrolysins. It has been thought possible to explain on a similar basis through the formation of syncytiolysins conditions of such an acute pathological character as eclampsia. How far these views are correct, it is at present impossible to determine. The possibility must, however, be conceded that the same principle underlies equally very striking adaptations and somewhat less apparent disease producing processes. In recognizing this it will be easier to apply to the phenomena of adaptation the same causative investigation as to other phenomena.

THE REGENERATION OF A DOUBLE CHELA IN THE FIDDLER CRAB (GELASIMUS PUGILATOR) IN PLACE OF A NORMAL SINGLE ONE.[1]

CHARLES ZELENY.

The double chela described in the present paper was regenerated from the distal end of the stump of the smaller chela of a male fiddler crab (*Gelasimus pugilator*) after autotomy of that appendage at the breaking joint. The case seems of interest not only because it is a regeneration product but also because it belongs to the rare class of truly double appendages.

The crab which developed the double chela originally possessed a normal small chela on the right side and a normal large chela on the left and was operated on at Cold Spring Harbor on July 2, 1902.[2] The nerves of the two chelæ were injured by a needle, the injury in each case resulting in immediate autotomy of the appendage at the breaking joint. The crab was isolated and fed on *Mytilus* (horse-mussel). It moulted on August 4, 33 days after the operation. On both sides the regenerated chelæ were of considerable size and the right or smaller one was seen to be double. The crab was kept under observation for 17 days after the moult, or 50 days after the operation. Two views of the chela are given in the accompanying figure which shows the terminal four podomeres. The first,[3] second and third podomeres are of the typical single form. The fourth podomere, however, is divided terminally so that there are two entirely separate indices (*I*, *I'*). The split extends well down into the body of the propodite on this side. In connection with each index there is a dactylopodite (*D*, *D'*). The split on the dactyl side of the propodite, though not as deep as on the index side, nevertheless

[1] "Contributions from the Zoölogical Laboratory of Indiana University," No. 67.

[2] The operation was performed as part of a series of experiments on compensatory regulation which are described in a paper now in press under the title of "Compensatory Regulation," *Journal of Experimental Zoölogy*, Vol. II., No. 1.

[3] Not shown in the figure.

completely separates the two dactyls and forms a very distinct groove in the propodite.

One of the resultant pinchers (*DI*) the one more directly in the line of the axis of the chela is stouter than the other. In the stouter chela the dactyl and index are approximately of equal length. In the smaller chela (*D'I'*) the dactyl is very noticeably longer than the index. Except for these slight differences and the more slender character of the smaller chela it is a perfect mirror image of the other. As the split is deeper on the index than on the dactyl side, the dactylopodites are nearer together than are the indices. The planes of the two chelæ

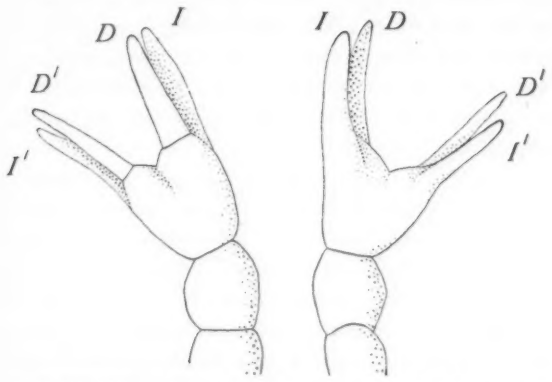

FIG. 1. Double chela of *Gelasimus pugilator* (× 12). Left figure: viewed from pollex border. Right figure: viewed from index border.

therefore converge slightly on the dactyl side and diverge on the index side. In life both parts of the double chela functioned as pinchers.

DISCUSSION.

The double chela which has been described evidently comes under the group of true double appendages, a group the existence of which Bateson[1] was loath to admit since he considered the possibility that the supernumerary appendage in such cases is in reality itself double, as is true in a great number of the abnormal limbs so far described.

[1] Bateson, "Materials for the Study of Variation," 1894.

The nearest approach to the condition of the present double chela that I have been able to find in the literature is the right cutting chela of a female lobster figured by Herrick on p. 147, plate E, of his "American lobster." This gives a chela which is apparently truly double from the carpopodite out. The two parts resemble each other very closely.

In Bateson's list[1] Nos. 832 to 835 come under the same head. Of these No. 833 (Fig. 200 1, p. 542) described by Faxon[2] as a right chela of *Homarus americanus* shows two dactylopodites articulating separately. The index is bifid at its tip and has two rows of teeth, one on each edge. If the separation of the dactylopodites had been slightly greater than shown in Faxon's figure and the split in the index had extended slightly further down on its side than the split in the dactyl a fair representation of the condition in the Gelasimus chela would have resulted. A similar modification in the degree of the split would bring Nos. 832, 834 and 835 into the same category.

There can be no doubt that the present regenerated double chela is a true double appendage, one part being a mirror image of the other except for the slight differences given above. The relation of the parts is such as one would expect if after the regenerating bud of the chela had become specified a mechanical force had partly separated it into two nearly equal parts by a quantitative split, *i. e.*, one passing in the plane of the future chela. With but a slight rearrangement of the materials in the two parts each is supposed to have been able to form a complete pincher in a manner entirely similar to that in the constriction experiments of Spemann and others[3] on Amphibian eggs by which double and partly double embryos were produced. The wider separation of the indices as compared with the dactyls is supposed to be due to the greater depth of the mechanical split on this side.

I have no suggestion to make as to the nature of the agency which caused the division of the embryonic mass. Spemann's

[1] *L. c.*, pp. 541, 542.

[2] Faxon, *Bull. of the Mus. of Comp. Zoöl. Harv.*, Vol. VIII., No. 13, 1881.

[3] Spemann, Hans, "Entwickelungsphysiologische Studien am Triton-Ei," *Roux's Archiv*, Bd. XII., p. 224, 1901. Herlitzka, A., *Roux's Archiv*, Bd. II., pp. 352 ffl. Endres, H., *Roux's Archiv*, Bd. II., pp. 517 ffl.

experiments on the production of double embryos by constriction of Triton eggs by means of hairs and similar experiments of others as mentioned above, certainly indicate the possibility of producing double chelæ by similar methods used on the regenerating buds of Crustacean chelæ. Such experiments will be undertaken as soon as suitable material can be obtained.[1]

If the explanation given turns out to be a true one it follows that the mass of embryonic cells which in the ordinary regenerating bud forms a single chela has because of the partial separation of its material been forced to develop a double one. Or stating the same facts in the convenient terms introduced by Driesch we may say that while the early regenerating bud has ordinarily a *prospective value* of a *single* chela the present case certainly shows that the same mass of cells has a *prospective potency* of *two* chelæ.

INDIANA UNIVERSITY,
February 8, 1905.

[1] Andrews, E. A. ("An Aberrant Limb in a Cray-fish," BIOL. BULL., Vol. VI., No. 2, January, '04) attempted to induce the development of monstrosities in the propodites of the chelate walking legs by making deep cuts in the adult limbs at the places where the new structures were expected to develop. Though 103 operations were made no positive results were obtained.

THE SPERMATOGENESIS OF SCUTIGERA FORCEPS.

GRACE MEDES.

INTRODUCTION.

In the present paper it is my purpose to note briefly some observations upon the spermatogenesis of *Scutigera forceps*. The spermatogenesis of *Chilopoda* has been the subject of a number of investigations during the last few years besides the earlier ones of Carnoy and Prenant. Carnoy, in his pioneer monograph on "Cytodiérèse Chez les Arthropodes," demonstrated the value of this material by a series of observations on *Geophilus*, *Lithobius*, *Scutigera* and *Scolopendra*, although many of his results have been disproved by later investigators. The most suggestive part of this work has to do with the various kinds of nucleoli and their composition and structure. He divided these bodies into four classes: *nucléoles nucléiniens*, *nucléoles plasmatiques*, *nucléoles mixtes* and *nucléoles noyaux*. In Chilopods, he found two of these: nucléoles noyaux in *Lithobius forficatus*, *Scutigera arachnoides* and *Geophilus;* and nucléoles plasmatiques in *Scolopendra dalmatica*.

Prenant worked upon *Scolopendra* and *Lithobius*, his results being very similar to those obtained later by P. and M. Bouin. These two,. in connection with R. Collin, have worked upon several forms of Chilopods, *Lithobius forficatus*, *Geophilus linearis* and *Scolopendra morsitans*. These authors have concerned themselves principally with cytoplasmic structures, although they have given some attention to nuclear structures as well. The large nucleolar bodies found in *Lithobius* and *Geophilus*, according to them, are of an achromatic nature, and would correspond more closely to Carnoy's nucléole plasmatique. The chromosomes are not derived from this body, but arise from granules scattered through the nucleus.

The results obtained by Meves and von Korff upon *Lithobius forficatus* are, in general, in accordance with those of Bouin. They take the same view in respect to the origin of the chro-

mosomes and regard in the same way the composition of the nucleoles.

Blackman ('01, '03) on the other hand, confined his work on *Scolopendra heros* principally to nuclear structures. According to him the large nucleolus found during the resting stage is almost entirely chromatic in nature, and from this body the chromosomes are directly derived at the opening of the active prophase. This structure, which he called the "karyosphere," would correspond to Carnoy's nucléole nucléinien.

Technique.

The animal from which my material was obtained is the black-banded Centipede, or wall-sweep—*Scutigera forceps*. The specimens were collected during the spring and early summer. The animals were killed and their testes immediately removed and placed in Gilson's aceto-nitric sublimate, where they were left for a length of time ranging from twenty-four to forty-eight hours. They were then removed, washed in running water for several hours, run through ascending grades of alcohol from 30 per cent. to 70 per cent., where they were treated with iodine solution to remove the crystals of corrosive sublimate, and preserved in fresh 70 per cent. alcohol. The material was embedded by the usual method in paraffin; sections varying in thickness from three to six micra were cut, fixed to the slide with albumen water and stained by one of the following methods:

The staining method productive of the most satisfactory results is Heidenhain's iron-hæmatoxylin. Sections were stained for observing different structures by varying the degree of extraction of the color. For some purposes, however, the sections stained with iron-hæmatoxylin and counter-stained with congo-red were more satisfactory. For the purpose of a micro-chemical test, Flemming's three-color method may be employed to great advantage. When stained in this manner, the different parts of the cell take on the following colors: the net-work stains a grayish purple, the centrosomes red, the chromatin in the dividing stages and in the karyosphere stains a transparent red, and in the diffuse condition found in the prophases and late telophase stains purple. The achromatic nature of the neucleoli is shown by the fact that

they stain scarcely more densely than the linin and assume a similar grayish appearance.

Observations.

Early in the spermatogonial stages, cells may be seen aggregated in separate groups which are surrounded by definite membranes, or cyst-walls (Fig. 3). The number of cells in these groups varies considerably — in those counted, from eight to over a hundred. This may be due to either or both of two causes. According to the general conception of a cyst, it is an aggregation of cells consisting of all the descendants of one primary spermatogonium. Observations supporting this theory have been afforded by St. George, '76 (*Rana temporaria*), Henking, '91 (*Pyrrhocoris apterus*), Toyama, '94 (*Bombyx*), Montgomery, '98 (*Pentatoma*), Paulmier, '99 (*Anasa*), McGregor, '99 (*Amphiuma*), and Sutton, '00 (*Brachystola*). However, from observations upon later stages, it is evident that in *Scutigera* these cysts are not such permanent structures. By a series of careful counts taken during the spermatogonial and early spermatocytic stages, it has been found that the number of cells in cysts in the same stage of development exhibits considerable variation. The counts of early spermatocytes shows them to exist in groups of from twenty-eight to forty. In the spermatogonia the results were somewhat different. In these, the highest number of cells found in one cyst was one hundred and eight, while the average number in cysts supposed to contain cells in the last spermatogonial division was about sixty. This, to my mind, can be due to but one cause: The cysts are not definite structures, but either by fusion cysts containing a larger number of cells are formed, or, by the division of one, two cysts containing a reduced number are produced. Sutton, in his paper on *Brachystola*, has estimated that the number of cells in one cyst at the close of the division period is two hundred and fifty six. In this case, all of the descendants of one primary spermatogonium are contained within one cyst, but in *Scutigera*, either this is not the case, or the descendants of one of these primary spermatogonia are much fewer in number. That the former is true rather than the latter, is indicated by the fact that in those cysts con-

taining the largest number of cells, all are in practically the same stage of development, and is further supported by the fact that, almost without exception, where cysts contain the smaller numbers, several composed of cells in practically the same stage of development are to be found in the same region of the testis.

The spermatogonia are small spherical cells, averaging about eleven or twelve micra in diameter. During the late prophase of the last spermatogonial division, the chromosomes lie in the clear nuclear area at the center of the cell (Fig. 1). These chromosomes, with the exception of one which stains more darkly than the others, present a dense granular appearance when stained with Heidenhain's iron-hæmatoxylin. The total number of these chromosomes is constantly thirty-seven (Fig. 1), which, if the accessory chromosome is excluded in both cases, is twice the number found in the spermatocytes, showing that this element remains distinct throughout the whole period of spermatogenesis.

During the metaphase of the last spermatogonial division the chromosomes may be seen lying in the equatorial plate, so placed as to form a ring; but owing to the minute size of the component elements and their close proximity to one another, they appear rather as a solid mass than as separate chromosomes (Fig. 2). When viewed in cross section, they present the appearance either of a plate densely crowded on the outside, with separate chromosomes scattered throughout the middle, or of a ring. The centrosomes may be seen lying in the cytoplasm at some distance from the cell-wall and at this period appear as minute particles staining an intense black with the iron-hæmatoxylin. Emerging from these points and directed towards the cell-wall are astral rays which are so fine and delicate as to appear mere elongations in the reticulum of the cytoplasm, rather than definite structures. The spindle-fibers, however, stain intensely and may be seen as separate threads connecting the chromosomes with the centrosomes.

In the early telophase, after the two groups of chromosomes have moved to opposite ends of the cell, the spindle threads, still staining intensely, lie stretched between these two masses, while the centrosomes have become lost (apparently) among the chro-

mosomes and the astral rays have disappeared. As the telophase advances, the chromosomes become more granular, stain less deeply, and appear in the next stage (Fig. 3), as a mass of dense granular threads. Already the growth period has begun, and a slight increase in the size of the cell may be observed. At this period one other important feature may be mentioned. This has to do with that peculiar structure, first clearly recognized by McClung ('99) as a chromosome, and described by him under the name of accessory chromosome. This element may be distinguished from the other chromosomes at this period by certain peculiarities in its form and behavior. During the telophase, while the other chromosomes have been lengthening into diffuse, flaky segments, this structure has remained unaltered and still retains the stain with the tenacity of a chromosome during the metaphase. As is to be expected from its composition, its reaction to stains is quite different from that of the ordinary chromosomes. When stained by Flemming's three-color-method, this element takes the saffranin, whereas the others retain the gentian violet.

During the period immediately following, the cell gradually increases in size, and the reconstruction of the nuclear membrane takes place. By this time (Fig. 4), the cell has increased about one half and the nucleus is considerably larger in proportion than at any other period. The cytoplasm, moreover, stains more lightly and shows scarcely any structure. The chromosomes, immediately after assuming their thread-like shape, lie tangled together in a close heavy mass (Fig. 3), but by the time the nuclear membrane has formed, they begin to spread out through this vesicle and the separate threads may be distinguished (Fig. 4). While this is taking place, these threads of chromatin have gradually become more granular and diffuse, and very evidently fewer in number, while the small black body, above pointed out as the accessory chromosome, has apparently become larger. Into the composite structure, hereafter to be called the karyosphere (Blackman, '03), the diffuse masses of chromatin gradually accumulate (Figs. 5–6) until the nucleus outside of the karyosphere is entirely free of chromatin, and only the linin net-work is to be seen.

Another noteworthy feature characteristic of this period is the presence of metaplasm in variable amounts within the nucleus. Immediately after the reconstruction of the nuclear membrane, when the dense mass of chromatin is beginning to become more diffuse, so that the presence of foreign matter could easily be detected, the nucleus never contains more than the one small round body, the accessory chromosome, beside the mass of thread-like chromosomes. But as the cell increases in size, others gradually appear in the nucleus, and less frequently in the cytoplasm. These bodies are homogeneous, and, like the karyosphere, take a dense black stain with the iron hæmatoxylin. In number they vary widely, some cells being apparently entirely free of them, others containing them in large numbers; while in size they range from scarcely distinguishable particles to large spheres. These bodies seem to be largest and most numerous during the mid-growth period. As the prophase approaches, they seem to decrease in size, and by the time the karyosphere breaks up, their remnants appear as mere particles scattered through the nucleus. Those found in the cytoplasm persist about the same length of time, although there they are much less common than in the nucleus. These bodies present in both nucleus and cytoplasm, correspond to the "ergastoplasmiques" of P. and M. Bouin, which are said to arise through the breaking down of the spindle fibers and which disappear at the opening of the prophase. Meves and von Korff found similar structures in *Lithobius*, but these are often present throughout all the stages of mitosis.

While these changes have been in progress, the cell has gradually been enlarging and by the time of the opening of the active prophase, it is many times its original dimensions. Fig. 7, which represents a cell of about average size, has an approximate diameter of seventy-five micra, while occasionally one may attain the enormous size of one-hundred micra.

Throughout this period, the karyosphere has maintained about the same appearance, excepting its increase in size. During the earlier stages, at the time when the chromatin was accumulating, it appeared to be sharply granular, and to take the stain more densely in some regions than in others; but as the growth period progresses, the karyosphere, in more darkly stained sections, shows

no differentiation of structure (Fig. 7). However, in sections two or three micra thick, from which the color has been sufficiently extracted, a careful study of the karyosphere under a high magnification, reveals the fact that it is not a homogeneous body, but is a complex mass, various parts of which react differently to the stain (Fig. 8). When colored with the iron hæmatoxylin, it appears to be composed of dark irregular masses, and between them smaller areas which stain brown and appear to be of a homogeneous consistency (Fig. 8, *b*, *c*, *d*). On the other hand, the larger masses are of slightly granular nature and commonly appear black. When the stain has been sufficiently extracted, however, they become grayish, and this color argues their chromatic nature, just as the brownish color without testifies to the achromatic nature of the karyosphere.

Evidences of vacuoles within the karyosphere may likewise be seen (Fig. 8, *b*, *c*, *d*) in thin sections lightly stained in the same manner, but their presence is shown much more clearly when in addition to the iron-hæmatoxylin congo-red is used (Fig. 8, *b*, *c*). These vacuoles in varying numbers may lie irregularly throughout the substance of the karyosphere.

From the growth period, the cell passes immediately into the active prophase. The first indication of this stage has to do with the appearance within the nucleus of the chromatic segments. As in *Scolopendra heros* (Blackman, '01), the first sign of activity is a very slight change in the structure of the karyosphere (Fig. 8, *c*). The texture of this body becomes more loose and the distinction between chromatin and achromatin becomes more marked (Fig. 8, *c*). This, however, is so slight that it would probably pass unnoticed were it not for the more striking phenomena which now follow. That the darker portions noted as occurring in the karyosphere at an earlier stage are chromosomes, appears very probable from the fact that they now seem to break off separately from its mass and to be directly transformed into short, thick, granular threads (Fig. 10).

It would naturally be supposed that, as the chromosomes leave, the karyosphere would become smaller, but such is not the case; even after a number of chromosomes are to be seen lying in the nuclear area the karyosphere is apparently as

large as before (Fig. 8, *g*, *h*, *i*, *j*). On close inspection, however, it may be seen that the vacuoles have greatly increased in size. Often these become united, and form one large vacuole at the center of the karyosphere, around which lie the remaining masses of chromatin (Fig. 8, *g*, *h*, *i*). When finally all these have emerged, there is left a mass of material staining more or less darkly, probably a true nucleolar portion of the karyosphere, and one small, round, dense black body, the accessory chromosome (Fig. 8, *i*). This latter presents exactly the same appearance as when last seen during the telophase, except for a slight increase in size, which may readily be referred to natural growth. The remaining mass, or true nucleolar portion of the karyosphere (fig. 8, *j*), now breaks up into small round bodies of more or less irregular size, which soon become indistinguishable from the remains of the metaplasm still lying in the nucleus during the growth period (Fig. 12, 13, 14). For this reason it is impossible to determine the ultimate fate of these bodies; for all seem to be of about the same size and appear to take the stain equally well.

Immediately after leaving the karyosphere, the chromosomes, as was shown above, shorten into dense granular cords (Fig. 9). These now undergo a longitudinal cleavage (Fig. 10), and the double thread of chromatin breaks up into a number of short portions of irregular size (Fig. 11). Whenever the position of these made counting possible, the uniform number of six in each half was to be observed.

Until the opening of the active prophase, the nucleus appears very clear, much more so than the cytoplasm, and contains a finely granular and irregular linin net-work, the meshes of which are much coarser than those of the cytoplasm. Metaplasm bodies left over from the preceding growth period may occasionally be seen caught in the meshes of this net-work, but with the exception of these and the karyosphere, no other structures are visible within the nucleus. Just preceding the emerging of the chromosomes from the karyosphere, however, the nuclear area becomes clouded over here and there with very thin, diffuse masses in which the segments of chromatin seems to become entangled as they pass out into the nucleus. It is these masses of achromatic matter which Bouin (*Lithobius forficatus*), Bouin an l

Collin, '01 (*Geophilus linearis*), Meves and von Korff, '01 (*Lithobius forficatus*) probably mistook for chromatin. In addition to the fact that the chromosomes may actually be seen emerging from the karyosphere, the achromatic nature of this substance is clearly shown by its staining reaction.

At first the chromosomes appear to lie loosely in these achromatic masses (Fig. 8), but as the threads of chromatin split, becoming shorter and thicker, the enveloping substance likewise contracts, staining constantly a darker brown, until when the thread breaks into the smaller fragments (Fig. 11) this envelops them and appears to hold them together. Soon these balls of chromatin become massed more closely together in more or less irregular shapes (Fig. 11, 12), but they always assume as a general outline, the typical form of the tetrad (Fig. 12). This particular shape is often obscured by the unequal sizes of the chromatin fragments, and the distortion of the enveloping sheath which, now being less diffuse, takes a considerably darker stain (Fig. 13). But in all cases favorable for observation, *i. e.*, such instances as when the whole body appears lying flat in one plane, the general outline of the tetrad is plainly discernible.

Thus it seen that while the tetrads in *Scutigera* present characteristics peculiar to themselves, still they are of the same general type found in other arthropods. The only detailed description of tetrad formation in Myriapods has been furnished by Blackman ('03), in *Scolopendra*. According to his description this occurs through a longitudinal and a transverse cleavage in the chromosome segments. The longitudinal division occurs first, and is followed by a bending of the two halves of the segments at their centers, giving the first indications of the second cleavage. "The short processes thus produced elongate at the expense of the quadripartite segment until a cruciform figure is produced, the four arms of which are of about equal length." In *Scutigera*, this process is naturally much obscured by the breaking up of the chromosome segments into the unequal fragments; but when these have again become united by the contraction of the segment, the resulting figure closely resembles that in a similar stage in *Scolopendra*.

As the chromosomes continue to contract, the smaller glob-

ules composing them become fused closely together, and from now on the chromosome always appears as a single structure (Fig. 13 *et seq.*). The enveloping sheath likewise continues to draw more closely about it, until it is finally indistinguishable from its contents (Fig. 15). By the time the last chromosome emerges from the karyosphere, those issuing first have already begun to undergo this operation; but, since each assumes the tetrad form very quickly after making its appearance in the nuclear area, presently all seem to be in an equally advanced stage of development (Figs. 12, 13, 15).

The history of the centrosome is one of the most difficult questions in the study of spermatogenesis of *Scutigera forceps*, on account of the peculiar nature of the cytoplasm. In structure this consists of an exceedingly fine and regular net-work, staining brownish with the iron-hæmatoxylin. A further difficulty is due to the presence in the cytoplasm of small bodies, probably of the same nature as the larger bodies mentioned above. These particles are indistinguishable from the centrosomes, except when the latter may be indicated with certainty by the surrounding astral rays; but these rays offer no sure solution of the problem, for they are of an extremely delicate nature, and endure for only a brief period. Carnoy encountered the same difficulty in *Scutigera arachnoides*, of which he says: "Les figures caryocinétiques y sont plus déliées et moins demonstratives" than in *Lithobius forficatus*, which he has already described as being hard to study on account of its having such dense and opaque cytoplasm.

Because of these difficulties, the centrosomes cannot be distinguished with certainty until the opening of mitotic activity. In the early prophase (Fig. 12), a pair of minute granules may be observed, situated in the cytoplasm at each side of the nucleus, about one third of the distance between its membrane and the cell-wall. These lie in a transparent clear space from which astral rays of exceeding delicacy extend out for a short distance into the cytoplasm, where they gradually become lost in the net-work. During the breaking down of the karyosphere and the formation of tetrads, these centrosomes remain stationary in the cytoplasm, and the asters maintain a nearly uniform size and strength.

The changes in the achromatic part of the nucleus during the progress of the prophases are as considerable as those in the chromatin. During the growth period the contents of the nucleus, consist of the karyosphere, metaplasm granules and a coarse linin net-work. The interspaces of this net-work are large and clear, contrasting decidedly with the thick yellowish appearance of the cytoplasm (Fig. 7). The appearing within the nucleus of the cloud-like achromatic substance, which later forms envelopes for the chromosomes, has already been described (Fig. 9). During this process, the net-work may be seen unbroken between these darker masses; but soon after the chromosomes commence to form, the threads of this net-work begin to coalesce and the meshes gradually become larger and more irregular (Figs. 10, 11, 12).

Thus we have the general appearance of the cell at about the mid-prophase (Fig. 12). The nucleus occupies the central position, while on each side, well out from its membrane, lies a centrosome. Each of these consists of two dark granules situated close together and surrounded by a very delicate aster. Within the nucleus the threads of the net-work have coalesced considerably and now form large and very irregular meshes. Several small, dark masses, either metaplasm or nucleolar part of the karyosphere, may be seen at various places in the nucleus. The chromosomes have split longitudinally, broken into small round unequal segments which have drawn together, and assumed the typical form of the tetrad. Around these, the achromatic masses which serve as envelopes, have contracted until no longer discernible.

The late prophase, judging from the comparatively few cells to be found in this stage, endures but a short period. The chromosomes continue to contract and to increase in density and staining ability until they are reduced to but a small fraction of their former size (Fig. 14, 15) when they lose their granular nature and stain an even, intense black. Immediately after continued contraction, they lose also the tetrad form (Fig. 16), and assume the shape of the "diplosome" described by Bouin (*Lithobius forficatus* and *Geophilus lineatus*). At the breaking down of the karyosphere, the accessory chromosome follows the

others into the nuclear area. At first it may be distinguished from them by its small, regular form and intense stain. But when the others have contracted into the dumb-bell shape, they are of uniform size with the accessory, stain similarly and thus are indistinguishable from it. But that this element assumes its position also in the equatorial area at the time of the metaphase is shown by the fact that there are nineteen chromosomes now present whereas but eighteen chromatin segments could be seen emerging from the karyosphere, and but eighteen tetrads observed later.

As the cell approaches the metaphase, the karyoplasm continues to break down, and assumes more and more the appearance of separate threads. At first these are wavy and irregular and often much branched, being, in fact, but segments of the linin reticulum. Later, however, at the time of the disappearance of the nuclear membrane, they straighten and lie like single threads in the cytoplasm. The centrosomes, meanwhile, have remained in the cytoplasm at some distance from the nuclear membrane. As this fades away, the threads formed from the network of the nucleus stretch from one centrosome to the other, and, losing their granular consistency, take on the form of distinct, definite fibers.

Preceding the breaking down of the nuclear membrane, the nucleus often wanders from its central position in the cell and assumes a position near the cell-wall. Bouin ('01), in *Geophilus lincaris*, describes two methods of cell-division; one "division axiale," which represents the usual type where the centrosomes lie at opposite poles of the cell with the spindle between; the other, "division laterale," in which the spindle lies near the periphery and is often tangent to the cell-wall. In the latter case, he says: "Les corpuscules centraux et les sphères s'attachent contre la face interne de la membrane cellulaire, la ligne que les réunit est souvent d'une longueur moindre que le grand diametre de la cellule . . . Les extrémités du fuseau peuvent se trouver si voisine des centres cinétiques qu'elles paraissent se continuer avec la substance de ces formations."

But although cells answering to this description are often to be found in *Scutigera* before the metaphase, there does not seem to

be two methods of division; for the more nearly the cell approaches the metaphase, the more nearly the spindle extends through its central axis.

The fibers of the spindle, when first formed, are short and the ends converge to a point in the centrosomes, which, at this period, do not lie upon the cell-wall, but retain their position in the cytoplasm (Fig. 16). However, the centrosomes gradually move apart and come to lie on the cell wall at opposite sides of the cell. Meanwhile, the chromosomes have taken up their position in the equatorial plate and the spindle fibers extend directly through the center of the cell. They do not now, however, extend from centrosome to centrosome, but lie free in the cytoplasm with ends toward these bodies. Their extremities, while converging somewhat, do not meet in a point. Blackman, '01 (*Scolopendra heros*), described a condition closely resembling this during the formation of the spindle. The centrosomes, however, are upon the nuclear membrane at the time it begins to disappear, but the formation of the spindle and its appearance in early stages closely resembles that found in *Scutigera*. As the centrosomes recede toward the cell-wall, the spindle fibers remain united at the same point (the "apical point") from which center of convergence parallel linin strands extend to the centrosome, while from around these latter emerge the astral rays. Meves and von Korff, '01 (*Lithobius forficatus*), describe conditions more closely resembling those found in *Scutigera* after the formation of the spindle is established, although during this process, the similarity is not so close. Before the disappearance of the nuclear membrane, the centrosomes, surrounded by the centrosphere, have already taken their place upon the cell-wall. During the metaphase, he describes them thus: "Die Spindelfasern liegen in einer hellen Substanz (wahrscheinlich Kernsaft) eingebettet. Nach den Polen zu konvergieren sie etwas; ihre Enden sind aber nicht mit einander vereinigt, sondern hören frei auf; diejeniger Strahlen um die beiden Central-körperpaare welche direkt auf die Enden der Spindelfasern zu verlaufen, treten mit diesen allem Anschein nach nicht in Kontinuität."

This mutual independence of the spindle-fibers and the astral rays in *Scutigera* is the more clearly shown by the great dis-

similarity in their structure and general appearance; for while the latter are very fine and delicate and do not appear to be separate threads, but resemble rather mere elongations in the network of the cytoplasm, the former are very heavy, stain an intense black, and present the appearance of thick wires, the ends of which may be distinctly seen lying free in the cytoplasm.

At the opening of the metaphase (Fig. 17), the two centrosomes lie at opposite poles of the cell, surrounded by the clear transparent area and the radiating astral rays. The spindle lies directly through the center of the cell, pointing toward the centrosomes but not extending to them. It consists of heavy, intensely staining fibers. At their extremities these converge, but do not unite. In the equatorial plate are the chromosomes, nineteen in number, not lying in a circle on the periphery of the spindle, but apparently scattered more or less irregularly in one plane through the equatorial plate. Division and separation of the chromosomes occur immediately, and the two groups soon come to lie at opposite ends of the spindle fibers.

As has been stated above, after the other chromatin has entirely left the karyosphere, the accessory chromosome separates from the nucleolar portion, which immediately breaks up into numerous small particles. When the nuclear membrane has disappeared, these particles are, of course, cast out into the cytoplasm. They are not dispersed at once throughout the whole cytoplasm, but in the metaphase, take up a fairly definite position at each pole of the spindle proper, midway between centrosome and equatorial plate. Thus, before the attractive force of the centrosomes has succeeded in pulling apart the chromosomes, these particles already lie in the cytoplasm beyond the extremities of the spindle fibers. Here they remain as long as the centrosome is discernible, but after the latter disappears they wander out throughout the cytoplasm and soon degenerate.

Each centrosome, from the time it first becomes visible, consists of two minute granules, surrounded by a transparent space from which the short and delicate astral rays emerge (Figs. 12, 17). But at this period, shortly following the separation of the chromosomes, each centrosome becomes surrounded by a small, spherical-shaped region (Figs. 18, 19), which, when the

cells are stained but lightly by the iron-hæmatoxylin, presents a grayish appearance. In the center of this space, side by side, lie two black granules. When the cell is stained a longer period with the hæmatoxylin, this mass becomes very dark like the centrosomes, and shows an irregular outline, appearing as if formed by the fusing of the converging astral fibers. P. Bouin ('03), in a recent paper under the title of "Centrosome et Centriole" calls this entire structure, consisting of the two granules and the surrounding sphere, the centrosome, and each individual granule, a centriole; using this method to prove that the centrosome is not a permanent organ of the cell. But he seems to have misunderstood the meaning which Boveri, the originator of these terms, applied to them. The centrosome, as described by the latter, is "Ein Körper, an den die Sphärenradien direkt herantreten, ist das Centrosome." He tells us that it is present at all stages of cell-division, and divides to form the centers of the daughter-cells. According to his description this may, in the course of development, enlarge gradually, and become more complex, until just preceding the metaphase it may take on the form of a rather large, well-defined sphere in the center of which one or more minute central granules or centrioles may appear. Later, he adds as a test for distinguishing this enlarged centrosome from the centrosphere, that through the latter *astral rays may be traced*, while the former shows no such differentiation. Bouin accepts Boveri's definition for the enlarged centrosome during the metaphase, but rejects his definition of a centrosome during the prophase and declares the two granules occurring at that time to be the centrioles. In this manner he tries to prove that the centrosome is not a permanent organ of the cell. That his assertion is not valid in the case of *Scutigera* is proved absolutely by the fact that at no time during the existence of the sphere surrounding the two granules does it appear sharply defined from the astral rays, but these seem to traverse it and radiate from the two central granules which are invariably discernible in those cells not too darkly stained.

After the chromosomes have reached their destinations at the extremities of the spindle-fibers (Fig. 19), the asters surrounding the centrosomes fade away and the centrosomes themselves dis-

appear. Either they lose their staining capacity or, having no surrounding rays to mark them, are indistinguishable from the numerous particles, the remains of the nucleolar portion of the karyosphere, massed at this point. The chromosomes, having reached the end of the spindle-fibers at first appear to be attached to them at their extremities (Fig. 19), but as the fibers at once begin to undergo degeneration and to become considerably shortened, the chromosomes soon lie free in the cytoplasm (Fig. 20). While attached to the fibers they lie massed together (Fig. 20), but afterwards they spread over a greater area (Fig. 21) and the separate elements may be seen. Each daughter-cell contains eighteen chromosomes, which are still dumb-bell shaped, but slightly granular and less intensely staining than before. One cell contains in addition the accessory chromosome (Fig. 21), still homogeneous in consistency and staining black. This element, on account of its resemblance to the other chromosomes (during the metaphase) could not be distinguished from them, but at this period may plainly be discerned as it retains the same form and staining capacity possessed throughout the metaphase.

During the period immediately following the reconstruction of the nuclear membrane takes place. This process is the most remarkable phenomenon in the spermatogenesis of *Scutigera*, but as it takes place much more slowly in the second mitosis, affording better occasion for the study of the separate steps, it will be discussed in connection with that division.

The period between the two divisions passes quickly, although the chromosomes undergo great changes in appearance. During the metaphase of the first division, as was stated above, they assume a dumb-bell shape and appear homogeneous (Fig. 17). Immediately after this division they again present the same form and appearance, although reduced in size. As the telophase approaches, they become somewhat more granular, but retain the same general outline. After the reconstruction of the nucleus has taken place, however, the chromosomes gradually become more and more granular until they lie spread through the nuclear area as diffuse masses. Their identity is not lost, although they retain no definite form. As the membrane again breaks down preparatory to the second division, they gradually contract and assume once more their dumb-bell shape.

The formation and appearance of the spindle fibers up to the time of the metaphase has been described above (Fig. 16). During this period they lie like heavy black threads in the transparent substance derived from the nucleus (Fig. 17), one fiber being attached to each chromosome and so distinct that it may be traced from one end of the spindle to the other. The net work throughout the cell, except in the immediate vicinity of the centrosomes, is of the same regular nature characteristic of the cell during the resting stage, and shows the same density marking it throughout the prophase. Around the centrosomes, however, the astral rays are beginning to elongate and strengthen. At the same time the net-work surrounding the spindle becomes more ragged, the cytoplasm through the equatorial plane lighter and freer from linin until by the time the chromosomes have reached their destination at the extremities of the spindle fibers (Fig. 19) the entire area in the vicinity of the spindle is clearer, and scarcely any trace of the net-work can be discerned. Gradually now the spindle fibers spread across the equatorial plane (Fig. 20). As those upon the outside approach the periphery of the cell, where the constriction of the wall has already commenced, they become shorter and weaker as their extremities become dissolved in the cytoplasm through which they are scattered. At no time are the astral rays strong nor do they ever consist of heavy distinct fibers like those of the spindle, but they appear much lighter and at the extremities become lost in the net-work of the cytoplasm. They extend between the centrosomes and the equatorial plate but they never cross in this region. Indeed, scarcely ever can they be distinguished this far, but fade into the network of the cytoplasm about half way from the centrosomes to the equatorial plane (Fig. 17). However, the whole cytoplasm of the cell, extending from the centrosomes at the poles to the constricting cell-wall at the sides, soon becomes transparent and entirely free from net-work, apparently indicating some influence of the centrosomes on this area (Fig. 20).

As the constriction of the cell-wall continues, the regular network of the cytoplasm begins to re-form about the periphery, and the spindle fibers lying in the equatorial region become pushed together until they form a sheaf-shaped bundle, the ends

of which extend far into the cells. The fibers are exceedingly numerous, but not so heavy nor so darkly staining as when attached to the chromosomes during the metaphase and directly following it. Considering the size of the spindle, its disintegration occurs in a remarkably short time, indeed it begins as soon as the chromosomes have reached the extremity of the spindle. Until this time, the fibers were extremely heavy and presented a smooth appearance. But now they gradually become fainter and more granular, while those at the sides appear more and more feeble, until they can scarcely be distinguished from the network of the cytoplasm (Fig. 20). By the time they are pushed in to form the sheaf-like bundle (Fig. 24), the separate threads are of extreme fineness and delicacy. As the cell-wall presses inward, drawing the spindle together, a row of darkly staining bodies forms on the fibers in the equatorial plane, and appears to fuse. Only those fibers on the periphery seem to be affected thus, so that these granules do not form a plate, but a band around the circumference of the spindle. The period between the formation of the sheaf and the disappearance of the fibers passes quickly, although there is a wide variation in the time at which this takes place. In some cases the chromosomes are still in the vesicular stage after the cells have separated and the spindle fibers become greatly reduced in strength and numbers (Fig. 22), whereas in other instances (Fig. 24), a complete reconstruction of the nucleus has taken place before invagination of the cell-wall is completed. However, in no case does the second division commence until the spindle has so completely broken down that no trace of it remains.

During this entire period, the centrosomes are not discernible, but in the next metaphase they have reappeared, one granule on the cell-wall at each pole, surrounded as in previous division by a centrosphere and radiating fibers. These present an appearance almost identical with those in the preceding mitotic figure and bear about the same relative strength to the spindle fibers. As the nuclear membrane breaks down previous to the second mitotic division, the chromosomes all resume the same dumbbell shape as in the telophase of the former division. The accessory chromosome again becomes lost among the others as they

now possess the same form and staining reaction that it has maintained thus far throughout its course.

The metaphase passes rapidly, and soon the chromosomes, as in the preceding division, come to lie at the extremities of the spindle, which also closely resembles that of the first spermatocyte. The chromosomes soon become detached from the ends of these fibers and lie free in the cytoplasm. Generally they are aggregated more or less closely in one region, near where the extremity of the spindle fibers lay, but often they become scattered irregularly through the central area of the cell.

And now an occurrence takes place which has not been previously described in Myriapods, nor am I acquainted with the phenomenon in the male cell of any form, although it is a common occurrence in the egg cell. Exception to this statement should perhaps be made regarding somewhat similar conditions reported by Meves in *Paludina* and *Pygæra*. In this work Meves ('03) reports a vesicular condition of the chromosomes in the telophase of both spermatocyte mitoses, but this may persist after the nuclear membrane is formed. The conditions in *Scutigera* are therefore more comparable to those manifested by various ova. There is no immediate formation of a nuclear-membrane, but each separate chromosome, as it disintegrates, becomes enclosed in a membrane of its own, thus forming a structure similar to a nucleus, but containing only a single chromosome (Fig. 25). The diameter of each of these is no greater than twice that of the chromosome itself. During the formation of these vescicles, the chromosomes become somewhat granular and often lose entirely their typical dumb-bell outline. They assume various arrangements in different cells ; sometimes they lie close together, often they are scattered throughout the central region of the cell. Immediately after forming, however, these vesicles begin to fuse with one another, gradually producing large vesicles which contain more chromosomes. There seems to be no definite order of union, for sometimes they join in pairs (Fig. 26) and these gradually come together, while at other times several unite, forming a large vesicle around which are clustered smaller ones (Fig. 27). This latter case seems to occur more frequently although the former is often met with.

As was suggested above, this process occurs in both spermatocyte divisions, but in the first, it takes place much more quickly than in the second, and thus offers fewer examples for study. In case of the first division (Fig. 22), these vesicles always lie close against one another, whereas at the close of the second division, they are often widely scattered through the cytoplasm (Fig. 25). In the first division, too, the nucleus immediately after fusing becomes spherical, and all traces of its vesicular condition are lost. But at the close of the second division, the nucleus, in place of at once becoming spherical, retains for a considerable period traces of its former condition (Fig. 28), and often loses them only when transformation into the spermatozoön takes place. The membrane surrounding the nucleus at first appears very thin, like that of the vesicles; but soon after fusion is completed, it seems to increase considerably in thickness, and resembles that of the first prophase.

The only case which I have found of an occurrence resembling this was reported by Sutton, '00 (*Brachystola magna*). The metaphase is passed without exhibiting any unusual phenonenon. But during the telophase, an occurrence somewhat similar to that found in *Scutigera* takes place. "Each chromosome, on reaching the pole, begins to disintegrate, and at the same time, reconstructs its share of the nuclear membrane as a closed vesicle about itself. Later, all these vesicles become intercommunicating at their polar extremities, with the exception of one, which remains absolutely independent throughout its entire existence. The chromatin of the ordinary chromosome becomes diffused evenly in the nuclear space, while that of the one in the separate vesicle (the accessory chromosome) is deposited upon the inner surface of its capsule."

The spindle of the second mitotic division is markedly different from that of the first, although in the metaphase the two bear a distinct similarity. During the telophase, the breaking down of the net-work of the cell in the equatorial region and the spreading of the spindle fibers across this area is essentially the same as in the previous division, except that the fibers here are considerably fewer. As a result, no prominent sheaf-like bundle is formed when the constricting of the wall occurs, but these

fibers lie like a few strands obliquely through the cytoplasm (Fig. 25, *et seq.*). As soon as the chromosomes reach their destination at the extremity of the spindle, the net-work of the cell commences to re-form, and, within a remarkably short period, all trace of division is lost from the cytoplasm.

Soon after the second division occurs, the centrosomes also disappear. In the case of the former division, it was stated that they either lost their staining capacity or became indistinguishable from the remains of the karyosphere massed in their vicinity. But in this instance, the former is shown conclusively to be the case, partly because of the fact that here there are no such granules with which they could become confused, and partly for the reason that for a considerable time the aster remains visible, although at the point from which the fibers radiate, no centrosome is to be seen (Figs. 25, 26).

I wish to express my gratitude to Dr. C. E. McClung under whose direction the work was done, and to Mr. M. W. Blackman, for assistance in supplying material and for many valuable suggestions.

Laboratory of Zoölogy, University of Kansas,
June 8, 1904.

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XPLANATION OF PLATES.

All drawings are from camera-lucida outlines, taken with a B. and L. one-twelfth inch oil immersion objective and one-half, three-fourth, 1 inch B. and L. oculars. In reproduction a reduction of one-sixth has taken place.

Explanation of Plate II

Fig. 1. × 2,000. Spermatogonium in late prophase, about the time of the breaking down of the nuclear-membrane. The chromosomes, 37 in number, are spread over the central region of the cell. All are of a slightly granular consistency, except one, the accessory chromosome, which is homogeneous.

Fig. 2. × 1,000. Division of the last spermatogonium. (*a*) Chromosomes arranged on periphery of spindle or (*b*) scattered across equatorial plate. (*c*) Division figures typical. Centrosomes at apices of spindle. (*d* and *e*) Early and late anaphases.

Fig. 3. × 1,000. Late telophase of last spermatogonium. All chromosomes except accessory have become granular and lengthened into threads. The accessory is still homogeneous.

Fig. 4. × 1,000. Early spermatocyte. Chromatin segments beginning to form the karyosphere. The remaining segments scattered over nuclear area, in the form of long, granular threads. Growth period commenced.

Fig. 5. × 1,000. Later stage. Karyosphere becoming larger. Remaining segments of chromatin spread over the nuclear area in the form of very diffuse masses.

Fig. 6. × 1,000. Chromatin almost all in the karyosphere. Metaplasm bodies spread through the nucleus in considerable quantities. A less amount in the cytoplasm.

Fig. 7. × 1,000. Cell of mature size ready for the prophase. Karyosphere shows no differentiation. Metaplasm bodies decreased in amount. Network in cytoplasm is finer and stains more densely than that of nucleus.

Fig. 8. × 1,500. Karyosphere as seen in various stages. (*a*) During early growth period. (*b*) Just preceding the active prophase. Shows slight differentiation. Several vacuoles present in the matrix of spongy chromatin. (*c*) Karyosphere of same stage, showing the chromatin massed in more definite areas. (*d*, *e*) Very early prophases. Chromatin has become less densely massed; vacuolar substance has increased in amount; and has aggregated into one or several large vacuoles. (*f*) Part of chromatin has left the karyosphere. (*g*) Later stage. But little chromatin remains within the karyosphere, and this is massed about the large central vacuole. (*i*) All chromatin has left the karyosphere except one chromosome, which is probably the accessory. (*j*, *k*) Stages in the disintegration of the karyosphere, after the chromatin has all emerged.

Fig. 9. × 1,500. Early prophase. Chromosomes just commencing to leave the karyosphere. Cloud-like masses of achromatic matter beginning to arise in the nuclear area.

Fig. 10. × 1,500. Later stage in prophase. Chromatin segments granular and in the form of split threads.

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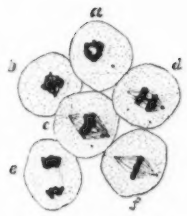

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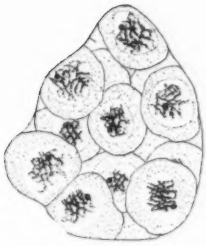

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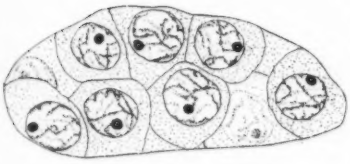

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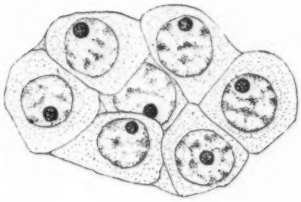

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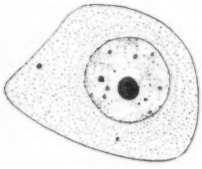

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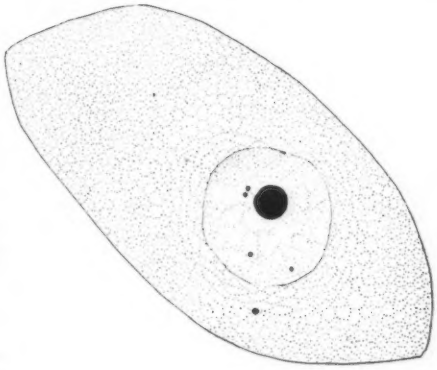

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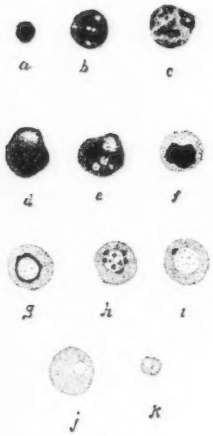

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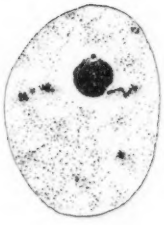

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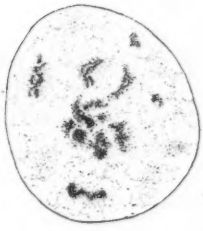

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Explanation of Plate III

Fig. 11. × 1,500. Later stage. Chromatin segments have broken into a number of small fragments. These are beginning to contract to form tetrads.

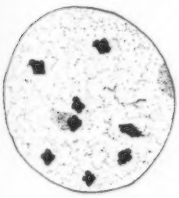
Fig. 12. × 1,500. Entire cell during mid-prophase. Chromatin in the form of cross-shaped tetrads. Centrosomes in the cytoplasm midway between nuclear membrane and the cell-wall.

Fig. 13. × 1,500. Later stage. Tetrads more contracted. Linin net work beginning to break down into more definite, less branched fibers.

Fig. 14. × 1,500. About the same stage. Tetrads in various stages of advancement.

Fig. 15. × 1,500. Later stage, shortly preceding the breaking down of the nuclear membrane. Chromosomes are nearly all homogeneous, but still preserve their tetrad outline. Nucleolar part of karyosphere still intact.

Fig. 16. × 1,000. Very late prophase. Nuclear membrane has disappeared and spindle is forming. Chromosomes have changed from tetrad to dumb-bell shape. Nucleolar remains of karyosphere grouped irregularly in the cytoplasm about the centrosomes.



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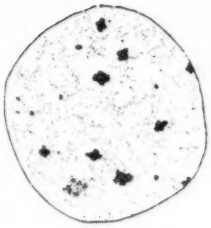

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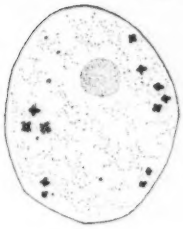

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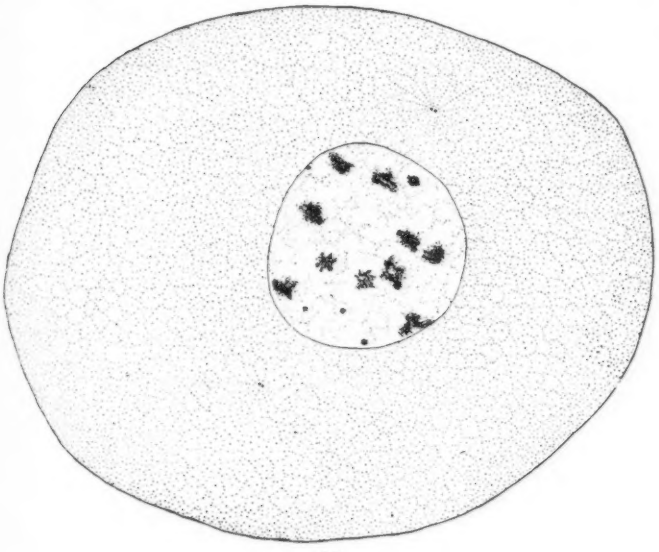

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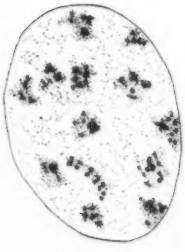

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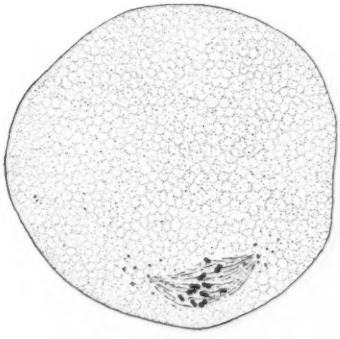

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Explanation of Plate IV

Fig. 17. × 1,000. Metaphase of first spermatocyte. Chromosomes grouped in equatorial plate. Centrosomes on cell-membrane. Spindle fibers do not extend to centrosomes, but lie free in the cytoplasm, their ends converging slightly. Nucleolar remains of karyosphere drawn to extremities of spindle fibers.

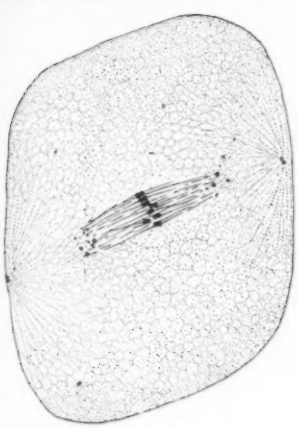
Fig. 18. × 1,000. Early anaphase. Chromosomes have divided, and again assumed their dumb-bell shape. Nucleolar remains of karyosphere drawn farther toward the centrosomes. Net-work still regular.

Fig. 19. × 1,000. Late anaphase. Chromosomes at poles of the spindle. Nucleolar remains of karyosphere drawn almost to cell-wall. Net-work beginning to break down in equatorial plate.

Fig. 20. × 1,000. Early telophase. Chromosomes massed at ends of spindle fibers, which have spread across equatorial region and begun to disintegrate at the extremities. Centrosomes have disappeared. Region from centrosomes to equatorial area considerably lighter, showing influence of centrosomes.

Fig. 21. × 1,000. Spindle forming into sheaf-shaped bundle. Chromosomes somewhat spread, becoming granular — except accessory, which is still homogeneous.

Fig. 22. × 1,000. Telophase of first spermatocyte. The vesicles in each one of which a chromosome has become enclosed are beginning to fuse.



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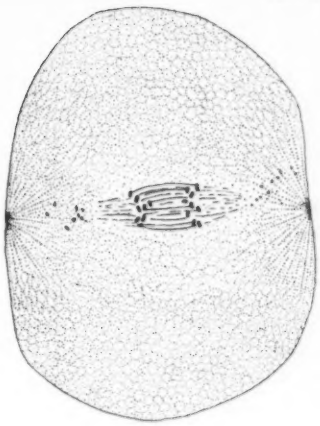

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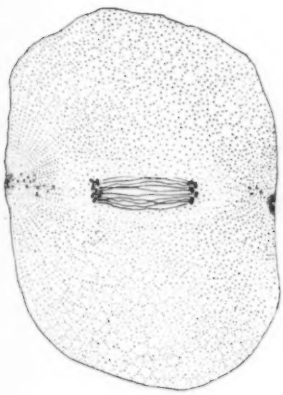

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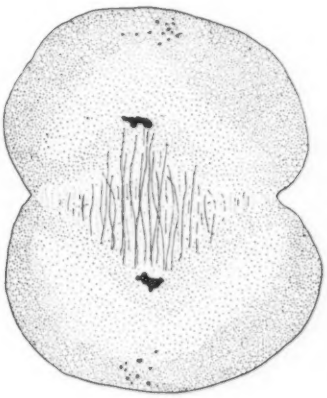

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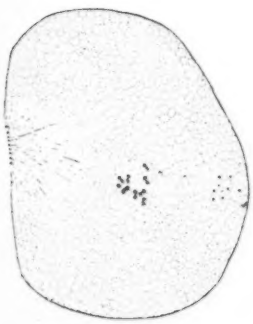

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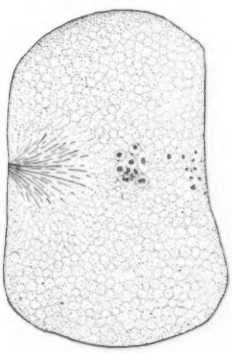

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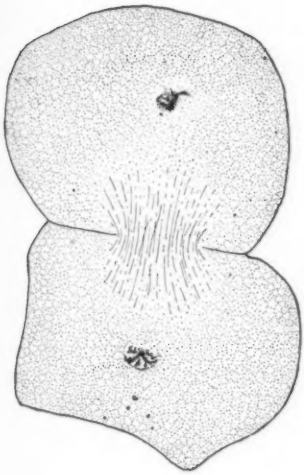
Explanation of Plate V

Fig. 23. × 1,000. Telophase. Nucleus just forming. Showing the slowness of the forming of the cell-wall in some instances. Compare with Fig. 22.

Fig. 24. × 1,000. Late telophase. Nuclear membrane formed. Chromosomes become diffused. Cell "A" shows accessory chromosome, which is absent in "B."

Fig. 25. × 1,000. Telophase of second spermatocyte. Each chromosome is enclosed in its separate vesicle. Centrosome has disappeared, but astral rays are still present. Spindle fibers persist as a few strands lying in the cytoplasm.

Fig. 26. × 1,000. Slightly later stage. Chromosome vesicles have begun to fuse.



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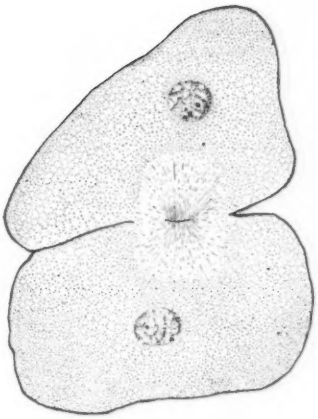

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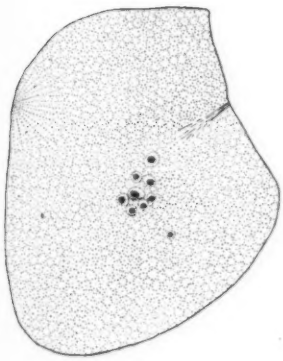

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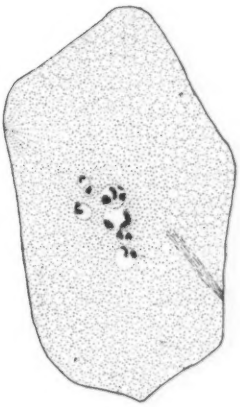

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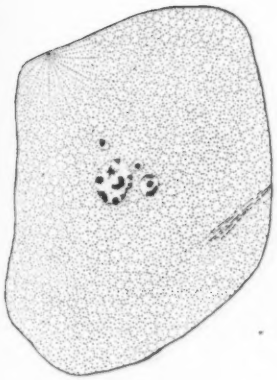
Explanation of Plate VI

Fig. 27. × 1,000. Still later stage. Fusion of chromosome-vesicles more advanced. Several of these have united, leaving part of the chromosomes still in separate vesicles.

Fig. 28. × 1,000. Fusion of the chromosome-vesicles complete, but the nucleus still retains a slight trace of its former vesicular condition. Nuclei of this shape are often to be found until transformation into spermatozoa takes place. All trace of astral rays has disappeared.

Fig. 29. × 1,000. All trace of vesicular stage has disappeared from the nucleus. Typical cell at the close of the second spermatocyte division.



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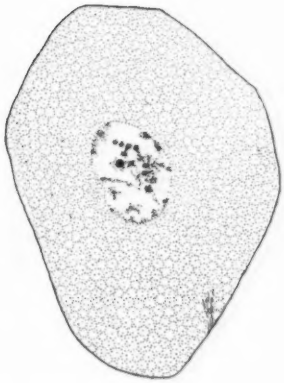

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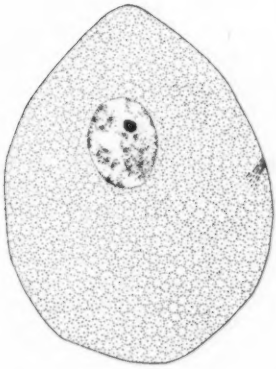

29

INCOMPLETE ANTERIOR REGENERATION IN THE ABSENCE OF THE BRAIN IN LEPTOPLANA LITTORALIS.

LILIAN V. MORGAN.

Previous experiments on the regeneration of planarians, more particularly of marine forms, seem to indicate that the presence of the cephalic ganglia is an important factor in regeneration. The following experiments were carried out to test the question further. The species used was a large marine planarian, *Leptoplana littoralis*, very abundant at Pacific Grove, Cal., found under wet stones just above the low-water mark.

Lillie[1] and Morgan[2] have observed in *Dendrocœlum*, that the power of regeneration is limited in posterior pieces, the regeneration anteriorly never being complete if more than about the anterior third of the worm is cut off. Schultz,[3] found in *Leptoplana* that anterior regeneration of a posterior piece cut at any level posterior to the cephalic ganglia is very slow and rarely if ever complete. Child[4] observed the same fact, and also in the case of lateral regeneration that "when they [the cephalic ganglia] are absent neither they nor the lateral part of the head removed are regenerated, though lateral regeneration of other parts may be more or less complete in the absence of the ganglia." In *L. littoralis* there is also a very marked difference in the rate and amount of regeneration of different kinds of pieces, and also in the behavior of the pieces.

Pieces of any size and shape, in which the cephalic ganglia remained, responded quickly to stimuli that affect the normal worm, and in general behaved like normal animals; moreover those that lived regenerated rapidly. Even small pieces (cut out

[1] Lillie, F. R., '01, "Notes on Regeneration and Regulation in Planarians," *Am. Journ. of Physiology*, Vol. XI., p. 129.

[2] Morgan, T. H.,'04, "Notes on Regeneration," BIOL. BULLETIN, Vol. VI., p. 159.

[3] Schultz, E., '02, "Aus dem Gebiete der Regeneration, II., Ueber die Regeneration bei Turbellarien," *Zeitsch. der wiss. Zool.*, 72 Bd., p. 1.

[4] Child, C. M.,'04, "Studies on Regulation, IV., Some Experimental Modifications of Form-regulation in Leptoplana," *Journ. of Exp. Zool.*, Vol. I., p. 95.

of the head with a straw), containing the eye-spots and cephalic ganglia and very little tissue besides, responded readily to light and touch, and crawled actively; in all cases in which such pieces survived, they quickly regenerated tissue in every direction, and in less than two weeks presented the appearance of small worms with heads very large in proportion to their tails (Fig. A, 7, 8). Pieces without the cephalic ganglia, on the other hand, behaved differently. They were sluggish in their movements, responded slowly and did not even stay under water, but were often found dried up on the sides of the dish. Also, as Child and Schultz found in the forms which they studied, anterior regeneration took place only to a very limited extent. Pieces cut at any level posterior to the ganglia often healed in such a way as to apparently prevent the growth of new tissue. After the worm was cut, the sides stretched very much and actually met in front of the worm, bringing the two halves of the cut surface in contact, so that they grew together, the point where they met in the middle line being raised above the level of the surface on which the worm crawled. To avoid such a closure of the wound, worms were in some cases cut so that the anterior end was pointed, or else the pieces were kept flat in vaseline under water. Even so, scarcely any anterior regeneration took place. When the posterior end of the same piece was also cut off, it was rapidly regenerated, proving, as in *Drendrocœlum*, that growth in the anterior direction was not wanting because of a general lack of power to regenerate in the absence of the cephalic ganglia. The pieces were not kept long enough to prove that anterior regeneration never could occur, but, whatever the limit, the rate is excessively slow as compared with the rate in other directions or in other sorts of pieces. One worm which was supposedly cut behind the brain, completely regenerated the anterior end including ganglia and eye-spots. It is undoubtedly true that it was cut posterior to the eye-spots, but the probability is that part at least of the ganglia remained. That this would be possible can readily be seen from the figure of a horizontal section of a normal worm, showing the relative positions of ganglia and eye-spots.*

* As this paper goes to press, a new paper has appeared by C. M. Child, "Studies on Regulation, VI., The Relation Between the Central Nervous System and Regula-

So far then the results showed nothing different from what had already been observed in other forms, but the power to regenerate ganglia had been tested only in pieces from which the tissue anterior to the level of the ganglia had been cut away, and the question remained whether that power was entirely wanting under all conditions. The ganglia were accordingly removed in a number of ways, all differing from a cut straight across the worm in that some of the tissue anterior to the level of the ganglia in each case remained in the piece that was to regenerate. The worms were cut as follows:

1. The ganglia were removed with as much of the tissue anterior to them as was necessary to make the cut, leaving however parts of the anterior end of the worm on both sides of the cut (Fig. A, 1).

2. The ganglia were removed from the side, with as little as possible of the lateral tissue and leaving all of the anterior tissue (Fig. A, 2).

3. The ganglia were removed in a right-angled piece, leaving the lateral part of the anterior end on one side (Fig. A, 3).

4. The ganglia were cut out with a straw, leaving a round hole in the worm (Fig. A, 4).

5a. The worm was cut in half longitudinally, and the ganglia removed from the pieces, or the ganglia were first removed with a straw, and the worm then cut (Fig. A, 5a). 5b. A slight variation of 5a was made by removing the ganglia with a straw, and a few days later, when the wound had closed, cutting the worm longitudinally (Fig. A, 5b). 5c. The anterior half of one side was cut off, and the ganglion of the other side cut out (Fig. A, 5c).

In all of these cases, unless as in a few instances the wound healed in such a way as to prevent growth of new tissue, regeneration took place, and externally, the worm appeared to be rapidly attaining the normal condition. The worms were not always kept until the form was completly restored, but regenera-

tion in Leptoplana: Anterior and Lateral Regeneration," *Jour. of Exp. Zool.*, Vol. I., p. 513, 1904. Among other results, he has found that anterior regeneration is complete when not much more than half of the cephalic ganglion is removed by a cross-cut.

tion was in every case rapidly taking place, and where checks were possible, pieces with and without ganglia were in the same condition. For example, in 5a, half-worms retaining one ganglion,

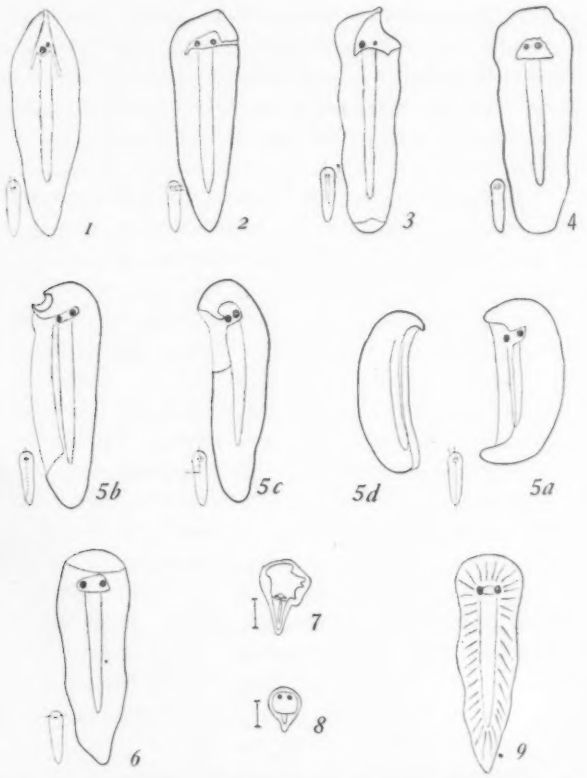

FIG. A, Diagrammatic figures of (1–6), worms regenerated after injury, indicated in each case by the accompanying small diagram. (7, 8), Regenerated pieces cut out of the head of the worm, and including cephalic ganglia and eye-spots. (9), Normal worm.

kept in the same dish with those without ganglia, regenerated at about the same rate.

In the pieces deprived of ganglia, the cut surfaces fused together, new tissue was formed, and eye-spots appeared in

about two or three weeks, often in two symmetrical groups, but sometimes with irregularities in size and position. The pieces at first were sluggish, and behaved like pieces from which the whole anterior end including the ganglia had been cut off, but as the eye-spots and other missing parts appeared the worms behaved more like the normal animal. In the light of what follows, it would be interesting to make more detailed observations on this point, for, in spite of the external signs of complete regeneration, sections show that the ganglia themselves have not regenerated. In the normal worm, the cephalic ganglia are well defined, and enclosed in a distinct sheath, and have the appearance in section of a brain of two hemispheres. The large nerve cords pass through the sheath and are connected with the ganglion (Fig. B, 1). In the regenerated worms, in place of the definitely defined cephalic ganglia, nerve fibers alone are present,

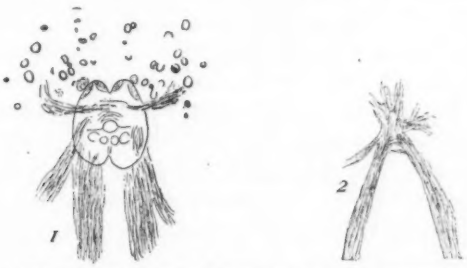

FIG. B (1), Diagrammatic figure of the cephalic ganglia, nerves and eye-spots of a normal worm, taken from a horizontal section. (2), Diagrammatic figure of the nerves in the position of the ganglia, in the regenerated worm of Fig. A (2), seven weeks after the removal of the cephalic ganglia.

which connect the large anterior and posterior nerve cords of the two sides of the body (Fig. B, 2). The eye-spots of the normal worm are more numerous and cover a larger area than in the regenerated worm. In the latter, the eye-spots are usually found in two well defined groups, though sometimes, crowded out of place or irregular, when the wound has healed in such a way as to leave too small a space for them to develop in the normal position.

In the absence of the ganglia it seems to be a fact that the presence of some anterior tissue is conducive to regeneration. It

will be noted that in all the cases described where regeneration occurred without the ganglia, some of the old tissue anterior to the level of the ganglia was left in the piece. The difference in regeneration with and without some anterior tissue was strikingly shown in the following experiment: the cephalic ganglia were removed with a straw, and the worm then cut in half longitudinally, the left half through an accident was injured, and some of the anterior tissue broken off. After seven weeks, the right half had regenerated well, and had for two weeks shown two distinct eye-spots, as in numbers of other similar cases. The left side, kept in the same dish for the same length of time, showed less regeneration, and no eye-spots had appeared, nor could any be found in sections (Fig. A, 5*a*, 5*d*). In all the cases where regeneration occurred in the absence of the ganglia, the cuts were made in such a way that lateral regeneration without anterior regeneration might account for the restoration of the form of the worm.

Regeneration of the anterior tip of the worm, that is when the worm has been cut off anterior to the ganglia, occurs in the absence of the ganglia as well as when they are present. The ganglia of several worms were removed with a straw, and after a few days, when the wound had closed, the anterior end was cut off anterior to the scar. Regeneration occurred as in check experiments, where the ganglia were retained, and by the time the eye-spots had reappeared, the form of the worm was completed (Fig. A, 6).

Summary and Conclusions.

The experiments show that:

1. Pieces containing the cephalic ganglia behave like normal animals, and regenerate readily.

2. Pieces deprived of the ganglia are sluggish, and may or may not partially regenerate.

3. Pieces cut across at any level posterior to the ganglia, do not regenerate to any extent anteriorly; but

4. Pieces deprived of the ganglia in such a way that some tissue anterior to the level of the ganglia remains in the piece,

regenerate partially. The normal form and behavior of the worm are restored, and the eye-spots are formed; but

5. The cephalic ganglia themselves never regenerate if completely removed.

6. Regeneration of the anterior tip of the worm occurs in the absence of the ganglia.

7. Lateral regeneration occurs in the absence of the ganglia, even though the ganglia themselves are not restored. It still remains unexplained why in the polyclads the tissue posterior to the cephalic ganglia does not regenerate the missing parts anterior to their level, and why the tissue around the ganglia does not restore them. It seems clear that the absence of the ganglia is in some way directly responsible for the lack of anterior regeneration, for when the ganglia are not entirely removed, complete regeneration occurs. The completion of the form of the worm and regeneration of eye-spots (though without the ganglia) in experiments 1–5 may have come about mostly through lateral regeneration, for in each case anterior tissue remained on one or both sides.

My work was carried out at the Marine Laboratory of Leland Stanford University. It gives me pleasure to express my appreciation of the courtesy extended to me while at Pacific Grove.

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